INVESTIGATIONS OF SOIL MICRO-HABITATS

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Clear statements have been made by various workers (Chesters, 1949; Garrett, 1951) regarding the importance of the micro-habitat in the ecology of fungi in the soil. Despite these, there still appear in the literature reports of indiscriminate isolations of fungi from soil, reports which provide species lists, but which give little or no indication as to the substrates in the soil from which the species are isolated or whether they are present as spores or mycelium.

The soil micro-habitats which can be studied with the greatest facility are those associated with plant roots and with fragments of plant débris, as these are easily separated from the mineral soil. Of these types of micro-habitat only the root region has been widely studied.

Washing techniques have been used by many workers for the study of active mycelia, particularly with reference to the root surface (Kürbis, 1937; Simmonds & Ledingham, 1937; Robertson, 1954; Harley & Waid, 1955; Stenton, 1958). However, despite the work of Chesters (1948) and Harley & Waid (1955), such techniques have not been sufficiently applied to other micro-habitats. We have applied variations of the Harley & Waid washing technique in the study of various types of plant débris on and in soil. The results given here refer to two types of decomposing plant material, pine-leaf litter and decomposing couch-grass material (*Agropyron repens*. Beaur).

Pine-leaf litter

Successions of fungi on naturally occurring plant débris, decomposing on the surface of the soil, have been studied by Chesters (1950), Mangenot (1952), Webster (1956, 1957), Hudson & Webster (1958) and Pugh (1958). One of the principal forms of such débris is the leaf litter of forest trees. This litter is the material from which the greater part of the organic horizon of soil is derived, and certain of its properties play dominant roles in determining the nature of the organic horizon (Handley, 1954) and in the development of the soil profile (Joffe, 1932).

The leaf litter of a pure stand of *Pinus sylvestris* is a very convenient

subject for study, as it has strong mor-humus-forming tendencies and the protracted breakdown process leads to a considerable accumulation of litter in progressive stages of decay which may be recognized as the 'litter', 'fermentation', and 'humification' sub-horizons (L, F, and H layers) as defined by Hesselman (1926). In the habitat chosen the accumulation is so great that a further sub-division of the 'fermentation' layer into F_1 and F_2 layers is clearly recognizable (Kubiena, 1953); F_1 needles being dark in colour and frequently still intact, and the F_2 needles greyish, fragmentary, and compressed together.

Most previous microbiological studies of podzols under pine species have either ignored the organic horizon altogether, or employed techniques such as the soil plate and the soil dilution plate, which are known to give an incomplete picture of the fungal population. Application of these techniques to the different layers of the organic horizon under consideration indicated a population almost entirely composed of heavily sporing moniliaceous forms such as *Trichoderma viride* and species of *Penicillium*. Microscopical examination of the H layer, however, showed the presence of large numbers of dematiaceous hyphal fragments, an observation previously made by Müller (1879) and Romell (1935). Many of these fragments were isolated directly by the method of Warcup (1955), but they invariably failed to grow.

This led to the belief that they were, at least in part, produced in the overlying layers of the organic horizon during the active decomposition of the litter, and persisted in the lower layers only because of their resistance to microbiological attack.

We have selected for active mycelia growing on or in the decomposing pine needles by applying a modification of the serial washing technique devised by Harley & Waid (1955) for the removal of detachable surface propagules. Batches of needles from each of the layers were shaken in 10 changes of a sterile 1% (v/v) aqueous solution of 'Teepol' detergent, followed by 10 changes of sterile water, prior to plating on 2% (w/v) malt agar. This technique was shown, by pouring dilution plates with samples of the washing water, to give satisfactory removal of spores from living needles and those of layer L, but was less effective for needles of the lower layers.

Mycelia growing in the interior of the pine needles were selectively isolated by surface sterilization of the needles with 0·1% (w/v) aqueous mercuric chloride solution before plating.

The results of a year's isolations obtained by the parallel application of these two techniques to the different layers of the organic horizon are shown in Fig. 1.

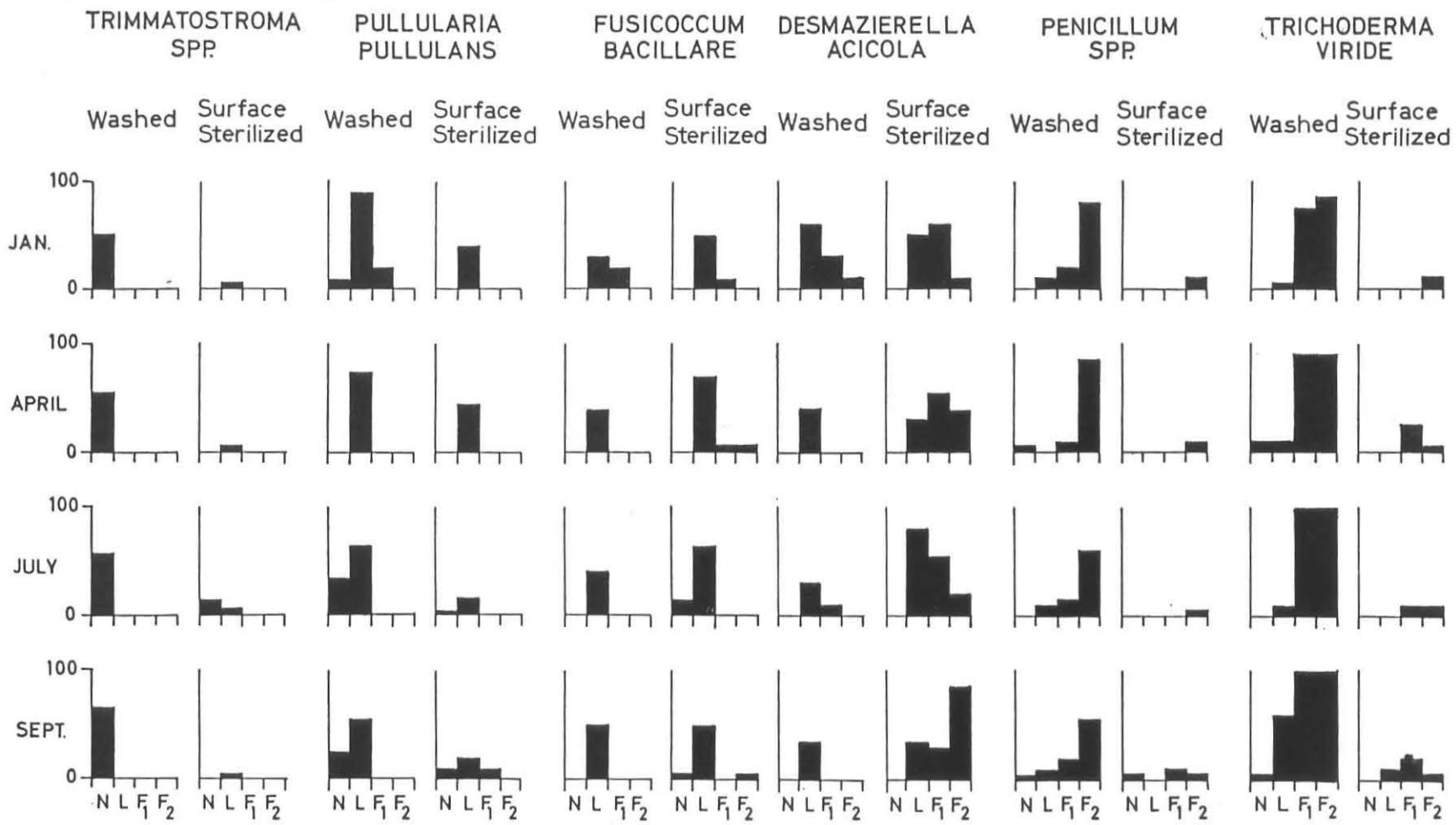

Fig. 1. Percentage occurence of frequently isolated fungi on washed and surface sterilized pine needles from the layers of the organic horizon (L, F_1 and F_2) and living needles from the trees (N).

Only the six most frequently isolated genera are represented. Of these, *Trimmatostroma*, *Pullularia*, *Fusicoccum*, and *Desmazierella* produce dark pigment. This is a very different picture from that obtained from dilution plates. *Desmazierella acicola*, perhaps the most important internal colonizer of the needles, was never isolated on dilution plates, and *Fusicoccum* was isolated only rarely by this method.

In addition to the cultural techniques, this particular micro-habitat lends itself admirably to direct microscopic observation, and this showed that at all stages of decomposition, even the specialized cultural techniques employed did not give a full picture of the active fungal population. Needles of the L layer were often seen to be colonized by the parasitic *Lophodermium pinastri*, which persists and fruits in the litter but does not grow in artificial culture (Jones, 1935). Numerous conidiophores of two hitherto undescribed dematiaceous hyphomycetes, *Helicoma monospora* and *Sympodiella acicola*, were seen on needles of the F_1 layer, and sterile dark hyphae and strands of Basidiomycete mycelium were observed in the F_2 layer. The two hyphomycetes and the sterile dark mycelium have been cultured by the direct plating of conidia or hyphae, but none of them was ever isolated by the normal cultural techniques described above. The Basidiomycete mycelia were not isolated on standard media.

These facts emphasize the importance of combining direct observation with cultural techniques if a fairly complete picture of the fungal population is to be gained.

Couch-grass debris

Here the soil under investigation was an acid sandy soil of low organic matter content. Samples of this soil were separated into two crude fractions—the mineral and the organic fractions. The organic matter consisted of fragments of dead plant material derived from couch grass.

The large organic fragments were subjected to serial washing with sterile water, after which pieces of organic matter (2 mm. long) were placed in nutrient agar. Throughout this group of experiments Czapek-Dox agar plus yeast extract and rose bengal adjusted to pH 5·0 was used as the medium for fungal isolations.

The crude mineral fraction was also washed serially, and fungi were isolated from the washed mineral material by the soil-plate technique (Warcup, 1950).

As well as the isolations from washed organic and inorganic matter, isolation of fungi from unwashed soil was accomplished by the normal application of the soil plate technique.

Table 1 gives data on the fungi isolated with the greatest frequency in these experiments. From this it can be seen that differences exist between the species complement of the washed mineral fraction, the washed organic fraction, and the unwashed soil. The main points may be summarized as follows:

(*a*) *Fusarium* spp. and *Cylindrocarpon* spp. are much more in evidence from plated organic fragments; in accordance with the results of other workers.

(*b*) Sterile mycelial forms are isolated with the greatest frequency from washed mineral material.

(*c*) Heavily sporing forms show a reduction in frequency of isolation from the washed fractions. This is particularly the case with the *Penicillia*.

(*d*) Certain members of the Mucorales (*Mucor hiemalis* and *Zygorrhyncus moelleri*) are isolated most frequently from washed mineral matter, but for the rest of the members of this order there is a decreased frequency of isolation from washed substrates.

(*e*) Isolates from washed organic fragments included several species not isolated from washed mineral material or from unwashed soil; for example, *Pythium* sp., *Cladosporium herbarum*, *Dicoccum asperum*, *Phoma* sp., *Microdiplodia* sp., and *Pyrenochaeta* sp. These occurred only rarely (with between 1 and 3% frequency), and have therefore not been shown in Table 1.

These results indicate that information regarding the distribution of fungi in soil will be given by the use of washing techniques; such information is not provided by many of the methods frequently used for the isolation of soil fungi. The results indicate that, for the most part, there is a decrease in the frequency of isolation of heavily sporing forms when washed material is plated. This may support the contention that many of the isolates are from mycelium present in the washed substrates.

This type of study is obviously only a preliminary step in soil micro-habitat studies. The organic and inorganic fractions used each represent groups of micro-habitats. The various plant parts from which the macroscopic organic matter of the soil is derived can often be easily recognized and separated, and dissection techniques on such material to allow more critical micro-habitat studies have been developed (Waid, 1956).

Separation of the mineral soil into fractions of known particle size can easily be achieved by sieving. This form of separation can easily be incorporated into a simple apparatus which will enable vigorous washing of the soil fractions. A modification of the apparatus described by Chesters (1948) has been used for this purpose. The sieving-washing

technique provides a practicable method easily adapted for dealing with many replicate samples.

It would seem that the use of washing techniques of the types described provide a quick and efficient means for the study of the active fungal population of soil and the distribution of these fungi. The hyphal isolation technique (Warcup, 1955) appears ideal for the study of fungi present as hyphae in soil. However, the hyphal isolation technique has limitations which have been given (Warcup, 1959); added to these there is the fact that the time taken for the picking and plating of hyphal fragments is considerable, and therefore the type of study for which this method is applicable is limited.

TABLE 1

Major fungi isolated from unwashed soil, washed mineral material and washed organic material

(*Figures represent % frequency of isolation*)

Fungi isolated	Unwashed soil	Washed mineral soil	Washed organic matter
Trichoderma viride	**81**	65	13
Penicillium spp.	**70**	7	10
Mucor ramannianus	**24**	18	—
Mortierella spp.	**23**	10	3
Ascomycetes	**10**	—	—
Sterile white forms	16	**60**	3
Mucor hiemalis	24	**55**	7
Zygorrhynchus moelleri	25	**40**	—
Sterile dark forms	1	**18**	4
Fusarium spp.	4	20	**50**
Cylindrocarpon spp.	—	—	**17**
Stemphylium sp.	1	—	**8**

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